George's PINK PAGES **November 2000**

Hygienic Behavior

I have written a great deal about the importance of new findings about bees for the 21st century, particularly PHEROMONES. However, and perhaps even more important to the beekeeper, who has been beleaguered with mites and now resistant American Foul Brood, is the "hygienic behavior" of his selected race or stock of bees. You know people who are always neat, tidy, and clean, and you really don't expect them to be "sickly", because they obviously use a lot of soap and water and keep away from garbage, refuse, and other sites that breed germs and disease. Conversely, there are those people that don't even bother to wash their hands after using the rest room, or don't protect a cut on their finger with an antiseptic and band aid.

There are very few things that we ALL agree on, but curtailing the use of chemicals to treat against tracheal and/or Varroa mites surely is one thing all of us would like. Based on the work of numerous bee scientists and researchers going back over 40 years, but gaining strong support during the past 5-6 years, I strongly believe that the use of hygienic bees will greatly diminish the need of any chemicals. You certainly are going to ask "Where do we get hygienic bees?" Right now, there are only two breeders who are advertising hygienically bred bees or queens: Heitkam's New World Carniolan field bred queens in the $10-$12 price range, and Glenn Apiaries Artificially inseminated Italian queens for $40. There has to be a major push or demand put upon the queen breeders of the country to modify their breeding procedures to select only those bees demonstrating "hygienic behavior" for them to use as larval sources for queen production. Your personal letters, a "drive" by American Beekeeper's Federation, as well as a "drive" by Eastern Apicultural Society, hopefully will get the reticent queen breeders "off their butt" and into the 21st century way of doing things. Of course, you know that all will hear from "Old George". The American Public would like more U.S. honey, the vegetable and fruit growers need more pollination of their crops, beeKEEPERS need bees that are not sickly, beeHAVERS need bees that don't have to be treated, and our bee breeders need the mental uplift that they would get from HEALTHY bees that don't need any chemical treatment that stresses the bee.

If you (and I hope you do) read Bee Culture, American Bee Journal, Speedy Bee, some states WEB sites, and George's PINK PAGES, you are going to see more and more articles about HYGIENIC BEES. This is your time to get on the "first floor" about the importance of hygienic behavior and its value to the bee itself, and the relieving of you "doctoring" your bees. BE INFORMED, BE A "KEEPER" RATHER THAN A "HAVER" of apis mellifera !

Although numerous famous scientists "toyed" with the term 'hygienic behavior', perhaps the principal exponents of "hygienic behavior" over 40 years ago were W. C. Rothenbuhler, S. Taber, and M. Gilliam. Although working independently of each other, their work was all pointed in the same direction, i.e., that certain stocks of bees who demonstrated quick and constant removal from the hive of "unclean" things like dead

brood, dead bees, wax cappings and all those things that might have pathogens, tended to be more resistant to disease than the stocks of bees who were not tidy or clean. Their work was aimed at the abatement of American Foul Brood and Chalk Brood. Many other researchers worked on this same assumption, but I don't want to name names in fear of slighting someone. How- ever, due to the lack of research funds, and indeed, the lack of interest of most benefactors, this work was "put on the back burner", until the "devilish" mites arrived just 15 years ago. Struggling to find bees resistant to mites as well as resistant to chemicals used in treatment for mites, initiated research again on "hygienic behavior".

How do bees demonstrate "hygienic behavior", or WHAT CHARACTERISTICS should be looked for? Sammataro and Avitabile write in their BEEKEEPER'S HANDBOOK the following: Nest cleaning activities include cleaning cells in preparation for egg deposition and keeping the nest free from debris and disease, as well as removing dead brood and even HEALTHY brood when there is a dearth of nectar or when the colony can no longer take care of the brood. Another nest-cleaning activity is coating the interior hive parts and the entrance with propolis .

Cells are polished (well-cleaned) before anything (eggs, nectar, pollen, or honey) are put in them. Cell preparation is accomplished by very young workers, only a few hours old. These young bees remove cocoon remains and larval feces from brood cells. The cleaned cells are then acceptable by the queen, who will lay eggs in them. Honey, nectar, and pollen will also be placed in these cleaned cells. Any remaining or uncleanable surface is covered by fresh wax or propolis.

Cleaning, or hygienic, behavior is a genetic trait, one that is desirable for beekeepers to perpetuate. For example, the continual quick removal of dead brood from the cells or the bottom board is considered hygienic behavior. Colonies whose workers demonstrate good hygiene are more likely to be free from some diseases, and such behavior may also help reduce mite levels.

Older workers take on the tasks of keeping the rest of the hive clean. Some examples are:

- Removing dead or dying brood and adult bees from the hive. Some bee colonies recognize varroa-killed brood and remove it.
- Removing debris such as pieces of grass of leaves as well as old comb and cappings.
- Removing granulated honey or dry sugar and moldy pollen.
- Coating the inside of the hive and wax cells with propolis.
- Propolizing cracks and movable hive parts, including bottom board and inner cover.
- Removing healthy drone brood or drone adults when the colony is starving.

Laidlaw and Page in their 1997 book, Queen Rearing and Bee Breeding, have a good bit to say regarding Hygienic Behavior in Chapter IX, The Genetic Basis of Disease Resistance. Beekeeping problems resulting from diseases and parasites are continually

becoming more abundant, costly, and difficult to control. Typically, the solutions have been chemical applications to hives with the inherent risks of contaminating wax and honey, and developing pesticide resistance in the target organism. The use of chemical reme- dies is also becoming less acceptable to the public, leading to increasingly more restrictions in their use, particularly around food products like honey. Therefore, in the future, breeding resistant stocks of honey bees will become increasingly important for maintaining a viable beekeeping industry.

The basis of all resistance to diseases and parasites is some mechanism whereby the host, the honey bee, defeats the disease agent. In order to select for resistance, there must be genetic variability for a mechanism. Genetic variability has been demonstrated for three general classes of disease resistance in honey bees: physiological, behavioral, and anatomical.

The best known example of disease resistance in honey bees is the hygienic behavior first described by O.W. Park in 1937. Apparently, there are two independent behavior- al activities: uncapping cells and removing diseased larvae from uncapped cells. These two activities were shown by Rothenbuhler in 1964 to be under the control of two independent genetic mechanisms. Hygienic behavior has also been shown to be an effective behavioral mechanism against chalkbrood disease and infestations of varroa.

There are remarkably few documented examples of controlled breeding for resistance to honey bee diseases and parasites. This is unexpected when considering the economic importance of honey bees, and the dramatic results obtained in those few cases where selective breeding was practiced. The first successful breeding program for resistance to AFB was implemented in 1934 by O.W. Park et al. Over the next 15 years, they successfully selected a resistant stock. The percentage of AFB innoculated colonies that became diseased dropped from 70% to 10% at the end of 5 years and to 0% t the end of 15 years! Park demonstrated that one mechanism of resistance was behavioral. He found some colonies quickly tore down and removed comb inserts containing scales of AFB that had been purposely paced there. Those hives that removed the diseased combs had a lower incidence of AFB disease. Park also demonstrated that the bees were responding to the presence of the disease, not just the foreign comb.

Rothenbuhler, after reviewing the work of Park, also believed that hygienic behavior alone was not sufficient to explain all resistance to AFB. As a consequence, he started a breeding program in 1954 specifically to study the potential genetic mechanisms of resistance. Using resistant and susceptible strains, Rothenbuhler demonstrated one behavioral, three physiological, and one anatomical mechanism. The results of studies of AFB resistance demonstrate the diversity of resistance mechanisms that can simultaneously occur when colony selection is used. Colony selection focuses on the occurrence of the disease, not the specific mechanisms responsible for resistance. Single factor resistance may occur if only a single mechanism is selected, e.g., hygienic behavior. However, multifactor resistance is probably more effective.

Colony infestation with Varroa mites is the most serious problem for beekeeping, worldwide. As a consequence, it is remarkable that there are no examples of successful artificial selection for resistance. However, much is known about the biology of varroa. This understanding of biology suggests potential mechanisms of resistance, some of which have demonstrated to vary genetically. An Example of Hygienic Behavior: apis cerana workers are able to detect capped brood cells that are infested with mites. They open the cells, remove, and kill the mites. apis mellifera workers apparently can also detect and remove varroa mites from infested brood cells in a way similar to apis cerana. If an infested cell is uncapped, the immature mites die, and the adult female mites must search for another larval cell, or are killed by the bees. This should slow the population growth of varroa mites in the colony.

The worldwide eradication of any honey bee disease is unrealistic. Therefore, in order to reduce economic damage, the beekeeping industry must depend on methods that maintain pathogens and parasites at reduced levels, now thought of as IPM, integrated pest management. The widespread use of chemical treatments has serious potential costs and risks resulting from the evolution of chemically resistant strains of disease agents, and the chemical contamination of hive products. Thus, the development of control methods that do not depend on chemicals should receive more attention. The evidence presented suggests that there is sufficient variability for resistance to diseases to make selective breeding a viable component of commercial honey bee management.

The work of Dr. Marla Spivak, Professor and Extension Specialist at the University of Minnesota is noteworthy. She has bred selected stocks of bees that can produce queens whose progeny exhibit habits of "hygienic behavior"; and these artificially inseminated Italian queens were made available through Glenn Apiaries in California this year, 2000. At this time, I do not know of any naturally mated queens for sale. Further, one queen breeder and package producer, Heitkam's Honey Bees, also in California, has been espousing and advertising "We are selecting for hygienic behavior".

Dr. Marla Spivak has given me permission to print out for you PINK PAGE readers a few paragraphs from her recent paper, The Hygiene Queen. Gary Reuter is a Research Technician who works with Marla.

THE HYGIENE QUEEN by Marla Spivak & Gary Reuter

Hygienic behavior of honey bees is the primary natural defense against American foulbrood (Park et al., 1937; Woodrow and Holst, 1942; Rothenbuhler, 1964) and chalkbrood (Gilliam et al., 1983). Hygienic bees detect, uncap, and remove diseased brood from the combs before the disease becomes infectious. Hygienic behavior also is one defense against Varroa mites (Peng et al., 1987), and although it is not the main mechanism of resistance to the mites (Harbo and Hoopingarner, 1997), it appears to limit their reproduction and population growth to some degree. Our studies have shown that it is possible to select for hygienic behavior without compromising honey production or gentleness (Spivak, 1996; Spivak and Reuter, in press). The trait can be found in approximately 10 percent of the managed colonies found in the United States, in any race

or stock of bees. We feel it would benefit the beekeeping industry to have hygienic lines of bees commercially available.

In this article, we present a simple way of screening colonies for hygienic behavior. We also discuss some frequently asked questions about the behavior, and how to breed hygienic colonies.

For years, we screened colonies for hygienic behavior by cutting out a section of comb (2 x 2.5 inches) containing sealed brood, freezing it for 24 hours, then placing the frozen comb section in the colony to be tested. If the test colony was hygienic, the bees would uncap and remove the freeze-killed brood within 48 hours when tested repeatedly (Taber, 1982; Spivak and Downey, 1998). Cutting comb sections out of frames is relatively messy and damages the combs, so we sought a better way of killing brood without having to handle the combs.

Dr. Jerry Bromenshank at the University of Montana was the first to suggest using liquid nitrogen (N2) to freeze a section of sealed brood within the frame. He found that freezing the brood this way was more efficient than cutting, freezing, and replacing comb inserts. Based on his suggestions, we conducted several tests to determine how much liquid N2 was necessary to completely kill the brood, and whether the test yielded the same results as cutting and freezing comb sections. We are now convinced that freezing brood with liquid N2 is the best screening procedure found to date for assaying hygienic behavior.

Frequently Asked Questions Often we are asked if hygienic colonies tend to have clean bottomboards, or if they tend to remove debris (such as wax paper, newspaper or cardboard) from the colony more quickly than other colonies. Mayer (1996) suggested that if colonies eat grease patties quickly, they might be hygienic. Removing debris from the hive is a form of cleanliness, but it is not necessarily a sign that the bees carry the hygienic trait.

Although the common usage of the word hygienic denotes cleanliness, hygienic behavior is a specific response by the bees to diseased and parasitized brood. A colony that keeps its hive clean does not imply that it will be resistant to diseases. Colonies must be screened for hygienic behavior using an assay such as the one described above. If a colony removes all of the freeze-killed brood within 48 hours, the colony will probably be resistant to diseases and will tend to remove mite-infested pupae. To determine whether they can actually resist the diseases or mites, the colony would have to be challenged with American foulbrood, chalkbrood or mites.

Another question we encounter concerns the difference between hygienic and grooming behaviors. Grooming behavior involves an interaction between adult bees; one bee removes mites or debris from the body of another bee. Alternatively, a bee may groom herself. Grooming and hygienic behaviors are different traits, and selecting for one does not imply selection for the other.

It is assumed by some beekeepers that hygienic behavior is associated with a high degree of defensive (stinging) behavior. This assumption stems from the reputation of the Brown line of hygienic bees studied by Rothenbuhler. Rothenbuhler (1964) showed that stinging behavior and hygienic behavior are inherited separately. Our experience has shown that hygienic colonies are as gentle as the stock from which they were bred.

Propagating Hygienic Colonies

Any race or line of bees can be bred for hygienic behavior. We recommend that bee breeders select for hygienic behavior from among their best breeder colonies; i.e., from those that have proven to be productive, gentle, and that display all the characteristics desired by the breeder. A breeder can get a head start on selecting for hygienic behavior simply by rearing queens from colonies that do not have chalkbrood.

Beekeepers should rear queens from unrelated hygienic colonies each year to avoid the negative effects of inbreeding. In time, if many bee breeders select for hygienic behavior, the frequency of the trait should increase in the general population of bees, which will increase the chances that any queen will encounter drones that carry the trait.

The effects of American foulbrood, chalkbrood and Varroa mites can be alleviated if queen producers select for hygienic behavior from their own lines of bees. Because a small percentage of the managed colonies today express hygienic behavior, it is important for many bee breeders to select for the behavior to maintain genetic variability within and among bee lines. Our experience has shown there are no apparent negative characteristics that accompany the trait. Years of research experience have shown it would greatly bene- fit the beekeeping industry if productive, hygienic lines were available commercially.

Marla is the Secretary of the American Association of Professional Apiculturists, of which I am a member; and both Gary and I are on the RESEARCH committee of the American Beekeepers Federation (and you should be a member of ABF, too). Beekeeping needs lots of research and research costs lots of money. YOU, beeHAVERS and beeKEEPERS need the ABF, and ABF needs your $25 for membership. Just ask ME, and I will send you the membership forms and my personal thanks!

I will end this now, hoping that you understand why I feel so strongly that honey bee stock that is selected because of its HYGIENIC BEHAVIOR might well be the solution to our present disease and pest problems and "freeing" the bees from the use of those NASTY chemicals. Unfortunately, my age coupled with the strokes I have already suffered probably will prevent my witnessing this great accomplishment, but at least I know right now that some scientists are actively including "hygienic behavior" as a highly desirable criteria for selection of breeder queens for queen breeders.